

Red Cabbage and Turmeric Extracts as Potential Natural Colors and Antioxidants Additives in Stirred Yogurt

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Received date: November 14, 2018; Accepted date: December 06, 2018; Published date: December 13, 2018

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Abstract

Background and Objective: The benefits of using natural colors in foods are many such as they are strong antioxidant, safe and get from renewable sources. Generally natural pigments do not cause any health problems; conversely, they may act as a health improvement. So, the objectives of this study are using natural colors and antioxidant from aqueous extracts of red cabbage and turmeric in the preparation of stirred yogurt. In addition, study the effects of these extracts on the quality characteristic of the obtained stirred yogurt.

Methodology: Four natural extracts were prepared by using only distilled water; red cabbage juice, aqueous extracts of anthocyanin, aqueous extracts of turmeric and aqueous extract of curcumin. The inoculated milk was divided into five portions, control and four treatments fortified with 10% of prepared natural color extracts. All samples were incubated at 43°C until full coagulation and the containers were transferred to the refrigerator overnight. The yogurt samples then stirred and stored in the refrigerator at 7° ± 1°C. Chemical, microbiological, antioxidant activity and organoleptic evaluations were carried out after that.

Results and Conclusion: The inclusions of red cabbage and turmeric extracts in the stirred yogurt resulted in products with appropriate and acceptable physicochemical, microbiological and sensory attributes. Aqueous extract of anthocyanin from red cabbage is the best extract, followed by the curcumin extract. These extracts were best known for their health benefits for humans and recommended to use in stirred yogurt and another dairy products.

Keywords: Red cabbage; Anthocyanin; Turmeric; Curcumin; Stirred yogurt; Physicochemical properties; Bacteriological properties; Sensory evaluation.

Introduction

Nowadays, the manufacturing of foods without using any additives is unthinkable both from the practical and theoretical viewpoint. The additives are used to raise the sensory attractiveness and product quality, also to confirm the correct track of the processing technology and storage. Colors are one of the most important additives in the foods industry and very important sensory attribute. Colors are used for adding or restoring the color of the food to boost its visual appeal and matching consumer expectations [1]. Currently, there is a big global trend for using natural colors in food manufacture, pharmaceutical, and cosmetics industries. The consumers awareness is increased towards natural products which from natural sources. Consumers prefer herbal medicines, natural foods and even in organic farming which do not use any pesticides or chemical fertilizers. People became afraid of food additives which are chemical synthesis; this is may be due to the profuse of using synthetic colors, chemicals, and derived products. Synthetic food pigments presently cause public anxiety concerning safety and the adverse impacts on human health; especially, the behavioral effects and neurological functions in children. So, the food industries were forced to replace them with natural colorants, which come from renewable and natural sources [2].

Red cabbage (*Brassica oleracea L.*) is one of the exceedingly famed healthy and nutritional vegetables which are consumed and eatable worldwide. The saleability of red cabbage is for its rich contents of phytochemicals, vitamins (C, E, A, K, B group), antioxidants, minerals (calcium, iron, manganese, magnesium and potassium), and low content of protein, cholesterol and saturated fats [3]. As well, red cabbage is best known for its high quantities of anthocyanin. The anthocyanin pigment color in red cabbage varies from reddish purple at low pH to intensive blue and green when the pH is high, which making it prevalent as a natural color dietary supplement in foods industries. These colored pigments consider vigorous pharmaceutical ingredients; many of their health properties have been discovered, such as potent antioxidants, chemopreventive, antimutagenic and anticancer. In the same time, these anthocyanins help in reducing the incidences of chronic diseases and preventing from diabetes and obesity, also preventing from cardiovascular diseases as well as improvement of vision [4]. Besides their color attributes, anthocyanin has played an important role as strong antioxidant more than famed antioxidants like butylated hydroxyanisole (BHA). The anthocyanin chemical structure is described by an electron deficiency, which extremely reactive towards ROS (Reactive Oxygen Species), also renowned as free radicals; therefore considered effective natural antioxidants [5]. Thus, there is growing interest in using anthocyanin of red cabbage as influential nutritional supplements in foods industries as a safe and functional antioxidant, and color additives. Also currently used in various dry mixes, candies, beverages, sauce, chewing gums and yogurts [3,6].

Turmeric (*Curcuma longa*) is a medicinal herb that contained polyphenolic compounds and known as a food colorant. Turmeric has been widely used for centuries in traditional medicine, because it has diversity therapeutic features including anti-inflammatory, antioxidant, anticarcinogenic, antiseptic, analgesic, and also exhibit antiviral activity. The biological characteristics of turmeric are attributed to curcumin content which densely exists in turmeric structure. Curcumin has different pharmacological effects; so it used in different applications such as a dyeing agent, antioxidant, antibacterial, anti-viral and anti-inflammatory. It was proved that curcumin is a potent agent in treatment and prevention of various cancers including lung, gastrointestinal, melanoma, breast, head, neck and neurological cancers. Furthermore, curcumin has been known as antioxidant; it contains a powerful mixture of antioxidant phytonutrients known as curcuminoids which inhibit cancer at different phases of tumor development, boosts colon health, provides neuroprotective activity and helps to maintain the health of cardiovascular system [7,8]. Food and Drug Administration (FDA) generally recognized it as safe (GRAS); and found that the healthy and safe dose of curcumin is up to 12 g/day for human consumption during the clinical trials without causing side effects [7].

Turmeric or curcumin has high considerable attention over the years according to its using as coloring agents in the food industries. Unequal synthetic pigments or dyes such as carmoisine and tartrazine that may macerate liver function and rise oxidative stress. Thus, these natural dyes are used not only as coloring for foods but also as a substance that boosts the health by preventing or healing many diseases [9].

The recent trends in food industries are the production of natural colored and flavored products using natural sources of bioactive compounds; also preparation in short time with extremely nutritive values and good quality of microbiology for human consumption. The benefits for using natural colorants in foods are many such as they are a strong antioxidant, safe, get from renewable sources, unsophisticated and harmonized with nature, their preparations contain a minimum potential of chemical reactions and also eco-friendly. Generally natural pigments or dyes do not cause any health problems; conversely, they may act as a health improvement.

So, the objectives of this study are using natural colors and antioxidant from aqueous extracts and juices of red cabbage and turmeric in the preparation of stirred yogurt. In addition, study the effects of these extracts on the quality characteristic of the obtained stirred yogurt.

Material and Methods

Fresh cow's milk used in this study was purchased from the herd of "The Animal Health Research Center", Cairo, Egypt.

Yogurt starter culture mixture of *Lactobacillus delbreukii* spp *bulgaricus* and *Streptococcus thermophilus* (1:1) was obtained from MIFAD Company (Misr Food Additives) Egypt. MRS agar and M17 agar were obtained from Merck, Germany. Red cabbage (*Brassica oleracea* var *capitata* L.) was purchased from a local market, Cairo, Egypt. Food grade turmeric powder was gained from "Imtenan Health Shop", Obour city, Egypt.

Preparation of red cabbage juice

The extracted juice of red cabbage was prepared as described by Chen et al. [3]. 1.6 kg of fresh red cabbage leaves were cut into small slices and pieces and then homogenized well with a blender. The homogenized pieces were extracted with 3 liters of distilled water in a big beaker using magnetic agitation for 16-18 hours at ambient temperature, then, filtered with Whatman paper No.1. The obtained juice was heated at 80°C for 15 minutes and kept in a colored glass flask at 7° ± 1°C until use.

Preparation of aqueous extract of anthocyanin from red cabbage: Aqueous extract of red cabbage anthocyanin was prepared as reported by Sahat et al., [10]. The pieces of fresh red cabbages were added in distilled water (1:1) in different beakers. These mixtures were stirred using a hotplate to heat at 35°C on for an hour until the purple color appeared. The extracted solution was then filtered with Whatman No.1 paper to remove any pieces of cabbage; and this process was replicated three times. In the third time of the replication, the specimen was soaked and stored in the refrigerator for 24 hours until all anthocyanin were fully extracted or the fiber become colorless by observation. The extracted solutions were collected and heated at 80°C for 15 minutes and kept in a colored glass flask at 7° ± 1° C until use.

Preparation of aqueous extract of turmeric

Aqueous extract of turmeric was prepared according to the procedure of Ibrahim and Abdel-hakim [11]. Water extract was obtained by adding 100 ml of distilled water to 20 grams of the turmeric powder in sterile wide-mouth screw-capped bottles (200 ml volume) and soaked for 24 hours at 5°C. The mixture was centrifuged at 3000 rpm for 10 minutes and filtrated through filter paper (Whatman No. 1), then the clear supernatant was collected. The obtained extract was heated at 80°C for 20 minutes in a water bath and cooled immediately in an ice bath until reaching 5°C then kept in a colored glass flask at 7° ± 1° C in the refrigerator until use.

Preparation of aqueous extract of curcumin from turmeric

Aqueous extract of curcumin was prepared according to the method described by Ibrahim and Abdel-hakim (2015) [11] 10 grams of turmeric powder was boiling in 100 ml distilled water, and left for full precipitation then filtrated using filter paper (Whatman No. 1); and the clear supernatant was collected.

Aqueous extract of curcumin was heat-treated at 80°C for 20 min in a water bath and cooled immediately in an ice bath until reaching 5°C and kept in a colored glass flask at 7° ± 1°C in the refrigerator until use.

Preparation of colored stirred yogurt

The stirred yogurt was manufactured according to the procedure of Mosiyani et al., [12] with some modifications. Fresh cow milk (12.5% total solids, 3.8% fat, 3.42% protein and 0.18% acidity) was heated at 90°C for 5 min and cooled immediately to 43°C. 3% of yogurt starter was added (a mixed culture of *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus* (1:1)). The inoculated milk was immediately dived into five portions, control and four treatments fortified with different natural color extracts as follow:

T1: Fortified stirred yogurt with 10% red cabbage juice,

T2: Fortified stirred yogurt with by 10% aqueous extracts of anthocyanin,

T3: Fortified stirred yogurt with 10% aqueous extracts of turmeric,

T4: Fortified stirred yogurt with 10% aqueous extract of curcumin.

All samples were filled in 100 gr container and incubated at 43°C until full coagulation and the containers were transferred to the refrigerator overnight. The yogurt samples then stirred and stored in the refrigerator at $7^{\circ} \pm 1^{\circ}\text{C}$.

Chemical, microbiological, antioxidant activity and organoleptic evaluations were carried out after 1 and 14 days of cold storage ($7^{\circ} \pm 1^{\circ}\text{C}$); all experiments were performed in triplicate.

Determination of total anthocyanin in red cabbage extracts

The total content of anthocyanin in red cabbage juice and aqueous extracts of red cabbage anthocyanin were determined according to the method of Rizk et al., [13]. A small amount from prepared extracts was diluted with distilled water (extracting solvent) to yield an optical density. The diluted extracts were kept in the dark for 2 h then the absorbance was measured at 520 nm. The total anthocyanin contents were calculated by using the following equation:

Total anthocyanin content (mg/100g fresh weight) = $\text{OD} \times \text{DV} \times \text{TEV} \times 100 / \text{SV} \times \text{SW} \times 51.56$

OD=Optical density

DV=Diluted volume for the O.D measurement

TEV=Total extract volume

SV=Sample volume

SW=Sample weigh in grams

51.56=E. value for which the major constituent red cabbage

Determination of curcumin content in turmeric extracts

Curcumin content in aqueous extracts of turmeric and aqueous extract of curcumin was determined by the described procedure by Geethanjali et al., [14]. 1 ml of prepared extract was taken and diluted with distilled water to 100 ml in a standard flask. The flasks were stored in dark conditions after covered the flasks with dark color paper because curcumin is light sensitive.

After 2 hours, the UV spectral readings for this solution were measured at 420 nm. The curcumin percentage in samples was calculated by the following equation:

Curcumin (%) = $[\text{Ds} \times \text{as} / 100 \times \text{Ws} \times 1650] \times 100$

Where Ds=dilution volume of the sample

Ws=weight of the sample taken in grams

As=absorbance of the sample

1650=standard value calculated by experts.

Color analysis of red cabbage and turmeric extracts

The color parameters for all prepared extracts of red cabbage and turmeric were measured as described by Ahmadiani et al., [15] by using a Hunter Lab Colorimeter Model D25A-2 (Hunter Assoc. Lab Inc. VA, USA). The obtained values were used to describe the following parameters:

L: lightness {black (0) to white (100)}.

a: redness (+) to greenness (-).

b: yellowness (+) to blueness (-).

Chemical analysis of colored stirred yogurt

The stirred yogurt samples were analyzed for moisture, fat, protein, ash, lactose contents and acidity (expressed as grams of lactic acid/100 g of sample) by the protocols of AOAC [16]. Whereas pH values of the yogurt samples were measured using a pH meter (using pH meter, Hanna Instruments, Italy).

Microbiological analysis of colored stirred yogurt

All the stirred yogurt samples were prepared for microbiological analysis as describing in the Standard Methods for the Examination of Dairy Products [17]. Enumeration of *Str. thermophilus* was carried out on M17 agar (Aerobic incubation at 37°C for 72 h) and *Lb. delbrueckii ssp. bulgaricus* on MRS agar (pH 5.2, anaerobic incubation at 45°C for 72 h). The plates were incubated in an anaerobic environment (BBL Gas Pak, Becton Dickinson Microbiology Systems). Log₁₀ cfu (colony forming unit)/g of sample was used to express the obtained results.

Antioxidant activity and total phenolic content of colored stirred yogurt

Antioxidant activity of colored stirred yogurt samples was determined as the procedure of Gulluce et al. [18]. The inhibition of free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) was calculated according to the following equation:

$\text{RSA}\% = (\text{Abs control} - \text{Abs sample}) / \text{Abs control} \times 100$

Total phenolic compounds were detected, using Folin-Ciocalleau reagent as reported by Zheng and Wang [19] and expressed as milligrams of Gallic Acid Equivalents (GAE) per 100 gram of dry weight.

Sensory evaluation of colored stirred yogurt

Sensory analysis was performed as reported by Januário et al., [20] with some modifications. The panelists asked to evaluate the acceptance of the formulations (appearance, flavor, color, texture and overall impression), using a 9-point hedonic scale (1=dislike extremely, 9=like extremely). The purchase intent was estimated using a 5-point scale (1=certainly would not buy, 5=certainly buy). The index of acceptability was counted by dividing the mean of the highest values obtained for each formulation.

Statistical analysis

All statistical analysis for means of all experiments was carried out using the SPSS 16.0 Syntax Reference Guide (SPSS, 2007) [21]. The gained results were expressed as least squares means with standard errors of the mean. Least significant difference (LSD) test was used to determine the statistically different groups ($p \leq 0.05$).

Results and Discussion

Physicochemical properties of prepared color extracts

Red cabbage is an important source for anthocyanin; and its colors are unique in being stable and potent in a wide range of pH with high

stability to light and heat compared to anthocyanin from other natural sources [13]. Anthocyanins are polar pigments high soluble in water than in non-polar solvents and this property help for extraction of anthocyanin by using 100% distilled water [22].

The average contents of total anthocyanin in aqueous extract red cabbage are shown in Table 1. The content of anthocyanin in aqueous extracts of anthocyanin significantly higher than red cabbage juice, it was 300 and 185.0 mg/100 g fresh weight, respectively. This may be due to that long time for the preparation of this aqueous extracts which

gave the opportunity to exit most of the anthocyanin from red cabbage leaves. The previous studies reported different contents of anthocyanin, as Wu et al. [23] found the content of anthocyanin was 322 ± 40.8 mg/100 g fresh weight, while Rizk et al. [13] and Ahmadiani et al [15] reported that the content of anthocyanin was 90.5 and 153 mg/100 g fresh weight of red cabbage, respectively. These variations of anthocyanin accumulation can be referred to the differences in red cabbage types and their development conditions as reported by Ahmadiani et al. [15].

Color extracts	Red cabbage extracts at pH 7.0				
	Total anthocyanin content (mg/100 g fresh weight)	Color of extract	Color characteristics		
			L	a	b
Red cabbage juice	185.0 ^B	Purplish	48.7 ^B	+10.4 ^B	-7.5 ^A
Aqueous extracts of anthocyanin	300.0 ^A	Reddish purple	59.1 ^A	+25.1 ^A	-6.7 ^A
Turmeric extracts at pH 3.7					
Extracts	Curcumin (%)	Color of extract	Color characteristics		
			L	a	b
Aqueous extracts of turmeric	4.6 ^b	Dark yellow	70.1 ^b	-6.9 ^a	+35.1 ^b
Aqueous extract of curcumin	7.0 ^a	orange-yellow	83.5 ^a	-8.1 ^a	+51.2 ^a

Table 1: Physical properties of colors extracts. L: black (0) to white (100), a: redness (+) to greenness (-), and b yellowness (+) to blueness (-). A, B, C... Values in the same column with different superscript small letters are statistically different (p ≤ 0.05). a, b, c : Values in the same column with different superscript small letters are statistically different (p ≤ 0.05).

Table 1 also showed the resultant colors and values of color parameters for red cabbage extracts. The obtained color for red cabbage juice was Purplish (extremely purple only), whereas the color of anthocyanin extract was reddish purple (violet-red). These differences in colors can be related to the extraction method and/ or the content of anthocyanin in each prepared extract of them.

The resulting values of "L" and "a" were significantly higher and "b" value was insignificantly lower in anthocyanin extract than the juice of red cabbage. This may be due to the high content of anthocyanin in the anthocyanin extract than juice; anthocyanins are known to be the most responsible for the color in red cabbage. These results in the same line with that reported by Alghamdi [24] who found that by increasing the anthocyanin content in red cabbage extract; the values of "L" and "a" parameters were increased, while "b" value was decreased; but Ahmadiani et al. [15] found different values for these color parameters. This variation in values for the same parameters because of many reasons, such as the method and the used solvent of extraction, the content of anthocyanin and plants cultivar.

The properties of the turmeric extracts are also presented in Table 1 Dissolving the turmeric powder in distilled water to prepare aqueous extracts produced acidic extracts (pH 3.70), as also reported by Surojanametakul et al. [25]. Curcumin is the major yellow bioactive compound in turmeric roots, so the turmeric content of curcumin defines its quality, color and therapeutic usefulness. Furthermore, curcumin is virtually insoluble in water at natural or acidic pH, and whereas it is soluble in alkaline solutions [25].

The content of curcumin in the aqueous extracts of turmeric was significantly lower than the aqueous extract of curcumin; this may be

as a result of using high temperature in the preparing curcumin extract. The resultant content of curcumin in the two extracts is in the same range of curcumin in previous studies, which reported that the concentrations of curcumin ranged from 0.3% to 8.6% in turmeric [26,27]. The content of curcumin and its quality in turmeric roots varies from sample to another depending on the seasonal, geographical area and type of soil, etc. [14]. The preparation of turmeric extracts by only distilled water introduced unclear extracts with dark and orange-yellow color, respectively; this is due to the low solubility of turmeric in water [28]. Moreover, the resultant values of color parameters showed that the curcumin extract had intensive yellow color (high 'b' value) than turmeric extract. The values of "L" and "b" were significantly higher in curcumin extract, whereas "a" value was lower than the turmeric extract. These obtained results are in agreement with De Lima et al. [29] who found that turmeric flour had higher "b" value and classified as orange-yellowish, than turmeric extract.

Physiochemical properties of colored stirred yogurt

The effects of adding aqueous extracts of red cabbage and turmeric on the chemical composition of stirred yogurt are presented in Table 2. Coloring the stirred yogurt with these natural extracts insignificantly affected the chemical properties of resultant stirred yogurt for total solids, fat and protein contents. Whereas, the lactose and ash contents were significantly affected by the addition of red cabbage and anthocyanin extracts only. The content of ash in all treated stirred yogurt was insignificantly higher than control, and this may be due the high contained of ash in all the used plant extracts. The colored stirred yogurt with red cabbage juice and anthocyanin extracts insignificantly

lower than control in total solids, protein and fat. These decreases are due to the addition of water extracts of red cabbage and anthocyanin, which contain a high percentage of moisture and very low total solids. Resulted in increased moisture content and decreased the total solid in

the resultant stirred yogurt. This interpretation has been mentioned previously by Damunupola et al. [30], who reported that the moisture contained in the juice of beetroot contributed to the total moisture content of resulting yogurt.

Treatments	Total solids	Fat	Protein	Lactose	Ash
Control (plain stirred yogurt)	13.22 ^a	4.25 ^a	3.79 ^a	3.49 ^a	0.89 ^b
Stirred yogurt colored with 10% red cabbage juice	13.09 ^a	4.08 ^a	3.65 ^a	3.13 ^b	0.97 ^b
Stirred yogurt colored 10% with aqueous extracts of anthocyanin	12.95 ^a	3.91 ^a	3.59 ^a	3.08 ^b	0.91 ^b
Stirred yogurt colored with 10% aqueous extracts of turmeric	13.60 ^a	4.28 ^a	3.88 ^a	3.54 ^a	1.24 ^a
Stirred yogurt colored with 10% aqueous extract of curcumin	13.42 ^a	4.07 ^a	3.81 ^a	3.44 ^a	1.15 ^a

Table 2: Proximate Analysis (%) of colored stirred yogurt. a,b,c,...: Values in the same column with different superscript small letters are statistically different ($p \leq 0.05$).

However, the lactose content in colored stirred yogurt with red cabbage and anthocyanin extracts was significantly lower than control; this may be due to high activity of starter bacteria in these samples. The differences between colored stirred yogurt with red cabbage juice and that colored with anthocyanin extract were slightly and insignificant, but the decreasing of lactose content in anthocyanin treatment can indicate to the high effect of anthocyanin on the activity and growth of lactic acid bacteria in the prepared stirred yogurt. These presented results were in the same line with Dimitrovski et al. [31]

On the other hand, using of turmeric and curcumin extracts as natural colors had a slight effect on the chemical compounds of resultant stirred yogurt. It can be seen that in Table 2. Colored stirred yogurt with turmeric and curcumin samples were insignificantly higher than control in total solids, fat, protein, lactose and ash contents. Turmeric and curcumin extracts contained a very small amount of fats and protein, which it cannot be considerable enough to affect in resultant stirred yogurt. One of the causes of high solids is that these extracts of turmeric were unclear and have very fine insoluble particles, due to the low solubility of turmeric in water. The other reason may be the high lactose content in these resultant colored stirred yogurt is the slight inhibitory effect of turmeric on starter bacteria; and this explanation was also reported by Fu et al. [32]

By the same way, the differences between colored stirred yogurt with turmeric and curcumin extracts were slightly and insignificant ($p > 0.05$), but the decreasing of lactose content in curcumin sample can indicate to the lower effect of curcumin than turmeric on the activity and growth of lactic acid bacteria in the stirred yogurt. Furthermore, the ash content in turmeric and curcumin treatments was significantly

($p \leq 0.05$) higher than control and red cabbage colored samples in, and this can be related to the high amount of minerals in these plant extracts. These findings are in agreement with Ibrahim and Abdelhakim [11].

Acidification and pH values of colored stirred yogurt during storage

During the manufacturing of yogurt, the main role of lactic acid bacteria is utilized and convert lactose into lactic acid; so the acidity increased and the pH decreased by the ending of the fermentation process. Moreover, the obtained values of acidity and pH varied from product to another depending on starter culture, milk composition and fermentation conditions.

The results regarding the effect of coloring stirred yogurt with natural extracts of red cabbage and turmeric on pH and acidity values during the storage at $7^\circ \pm 1^\circ\text{C}$ are summarized in Table 3. As shown from the results, coloring and addition of red cabbage juice and anthocyanin extract to stirred yogurt decreased the pH and increased the acidity significantly ($p \leq 0.05$) than control and turmeric treatments when fresh and after storage two weeks. These results are consistent with previous results obtained for lactose content. The reason for this is the fact that the addition of red cabbage juice and anthocyanin extract enhanced the metabolic activity of starter lactic acid bacteria in stirred yogurt, thus elevating the acidity. These reached results are in accordance with that of Dimitrovski et al. [31] who reported the ability of lactic acid bacteria to grow is better in the presence of red cabbage extracts.

Storage period (days)	Treatments				
	pH values				
	Control (plain stirred yoghurt)	Stirred yogurt colored with 10% red cabbage juice	Stirred yogurt colored 10% with aqueous extracts of anthocyanin	Stirred yogurt colored with 10% aqueous extracts of turmeric	Stirred yogurt colored with 10% aqueous extract of curcumin
Fresh	4.63 ^{Aa}	4.54 ^{Ab}	4.51 ^{Ab}	4.67 ^{Aa}	4.64 ^{Aa}
14	4.54 ^{Ba}	4.48 ^{Bb}	4.46 ^{Bb}	4.58 ^{Ba}	4.57 ^{Ba}

Acidity (% lactic acid)					
Fresh	0.87 ^{Bb}	0.94 ^{Ba}	1.06 ^{Ba}	0.82 ^{Bb}	0.85 ^{Bb}
14	1.03 ^{Ab}	1.21 ^{Aa}	1.35 ^{Aa}	0.97 ^{Ab}	0.98 ^{Ab}

Table 3: Acidity and pH values of colored stirred yogurt during storage at 7° ± 1°C. A, B, C... Values in the same column with different superscript small letters are statistically different (p ≤ 0.05). a,b,c: Values in the same row with different superscript small letters are statistically different (p ≤ 0.05).

In contrast of that, the addition of turmeric and curcumin extracts had insignificantly higher pH values, and insignificantly lower acidity values than control and red cabbage treatments in fresh stirred yogurt samples and at the end of storage time. These findings were in accordance with the lactose content of these samples and previously explained by Fu et al. [32]. However, there is insignificant dereferences between the extract of turmeric and curcumin extract.

Moreover, the acidity of all samples increased and pH decreased significantly during cold storage (p ≤ 0.05), due to the persistence of the *L. delbrueckii subsp. bulgaricus* in producing lactic acid during refrigerated storage, which known as post acidification as reported by Lucas et al. [33].

Bacteriological properties colored stirred yogurt during storage

The data of the viable counts of yogurt starter bacteria in the control and colored stirred yogurt were presented in Table 4. The viable counts of *Streptococcus thermophilus* were higher than *Lactobacillus delbrueckii spp bulgaricus* in all samples when fresh until the end of storage period. Seelee et al. [34] explained that during the fermentation, *S. thermophilus* produced lactic and formic acids to activate the growth of *Lb. delbrueckii spp bulgaricus* which responsible for producing diacetyl and acetaldehyde the main flavor compounds in yogurt. In the same trend, Dimitrovski et al. [31] found that the counts of *Streptococcus* were significantly (p ≤ 0.05) higher than *Lb. delbrueckii spp bulgaricus* in yogurt samples.

Storage period (days)	Treatments				
	<i>Streptococcus thermophilus</i>				
	Control (plain stirred yoghurt)	Stirred yogurt colored with 10% red cabbage juice	Stirred yogurt colored 10% with aqueous extracts of anthocyanin	Stirred yogurt colored with 10% aqueous extracts of turmeric	Stirred yogurt colored with 10% aqueous extract of curcumin
Fresh	7.8 ^{Ab}	8.9 ^{Aa}	9.2 ^{Aa}	7.2 ^{Ab}	7.5 ^{Ab}
14	6.5 ^{Bb}	7.7 ^{Ba}	8.3 ^{Ba}	6.1 ^{Bb}	6.2 ^{Bb}
<i>Lactobacillus delbrueckii spp bulgaricus</i>					
Fresh	7.2 ^{Ab}	8.0 ^{Aa}	8.4 ^{Aa}	6.6 ^{Ab}	6.9 ^{Ab}
14	5.9 ^{Bb}	6.5 ^{Ba}	6.8 ^{Ba}	5.5 ^{Bb}	5.8 ^{Bb}

Table 4: Viable cell counts (log10 cfu*/ml) of bacterial starter strains in colored stirred yogurt during storage at 7° ± 1°C. *Colony forming unit. A,B,C... Values in the same column with different superscript small letters are statistically different (p ≤ 0.05). a,b,c: Values in the same row with different superscript small letters are statistically different (p ≤ 0.05).

It can be seen that the addition of red cabbage juice and anthocyanin extract in the stirred yogurt significantly increased the viable counts of both yogurt starter bacteria than control and turmeric extracts in fresh time and the end of storage period. This is can be due to the ability of red cabbage and anthocyanin extracts to stimulate the growth rate of yogurt culture bacteria; and also the starter bacteria grew better in anthocyanin extract than red cabbage juice.

Dimitrovski et al. [31] and Semjonovs et al. [35] reported that the yogurt starter bacteria extensively grew in the cabbage juice and yield high viable counts, and actually cabbage juice enhanced the growth of lactic acid bacteria during the storage time.

On the other side, the lactic acid bacteria counts in the stirred yogurt which colored by turmeric and curcumin extracts were insignificantly lower than control. This may be due to the effect of

some turmeric components like phenols; which had an inhibitory effect on lactic acid bacteria. Also the inhibitory effect of the turmeric extracts was slightly high than curcumin extract; this is due to the presence of different types of phenols in turmeric extract than curcumin extract. These findings are in agreement of those obtained by Rahmatalla et al. [36] who mentioned that supplementation of yogurt with 0.75% (w/w) turmeric powder caused a reduction in total viable bacteria.

Table 4 also showed that the both of **lactic acid** bacteria in colored stirred yogurt approximately exhibited the same behavior in plain stirred yogurt. The counts in all treatments increased during the first week (data not shown) and then decreased significantly (p ≤ 0.05) until the end of the storage period. This may be due to the inhibition of the growth of bacteria as a result of continued acid production. The same

observations were found by Dimitrovski et al. [31] and Rahmatalla et al. [36].

It is worth mentioning, there is a consensus between the obtained results for lactose content, acidity and viable counts of starter bacteria in colored stirred yogurt samples. These results indicated to the improvement effect of red cabbage and anthocyanin extracts on the preparation of stirred yogurt, while turmeric and curcumin extracts had a somewhat inhibitory effect on it.

Antioxidant properties of colored stirred yogurt

The fermentation process of milk in the manufacture of yogurt decomposes some molecular and releasing different compounds such as fatty acids, peptides, organic acids and free amino acids which have antioxidant capacity. So the fermentation boosts the antioxidant properties in the dairy products. Currently, there is an increasing interest to enhance the antioxidant capacity of yogurt by incorporating natural extracts from fruits or vegetables or medicinal plants which are rich sources for valuable bioactive compounds and can potentially improve the antioxidant status for yogurt products [37].

The antioxidant activity and total phenolic compounds of colored stirred yogurt are presented in Table 5. The values of free radical scavenging activity (RSA) were found significantly ($p \leq 0.05$) lower in the plain stirred yogurt than the colored samples. The incorporation of color extracts from red cabbage which contain anthocyanin; and turmeric which contain curcumin significantly ($p \leq 0.05$) enhanced the antioxidant capacity of stirred yogurt. It can be seen that there were insignificantly ($p > 0.05$) differences between red cabbage juice and anthocyanin extracts in improvement the antioxidant of colored stirred yogurt; which confirms that anthocyanin is the most responsible compound for the antioxidant activity in the red cabbage. These results of RSA in stirred yogurt with red cabbage extracts were in the same trend of Rizk et al. [13] who mentioned that, the red cabbage compositions displayed twice in vitro radical scavenging activity than commercially antioxidants such as α tocopherol and butylated hydroxytoluene.

Treatments	* RSA (%)	**TPC (mg 100 g Gallic Acid Equivalents/100 gram)
Control (plain stirred yogurt)	22 ^d	113 ^D
Stirred yogurt colored with 10% red cabbage juice	36 ^c	210 ^C
Stirred yogurt colored 10% with aqueous extracts of anthocyanin	42 ^c	250 ^C
Stirred yogurt colored with 10% aqueous extracts of turmeric	75 ^a	435 ^A
Stirred yogurt colored with 10% aqueous extract of curcumin	62 ^b	310 ^B

Table 5: Antioxidant properties of colored stirred yogurt. * Free radical scavenging activity **Total phenolic content A, B, C... Values in the same column with different superscript small letters are statistically different ($p \leq 0.05$). a,b,c: Values in the same column with different superscript small letters are statistically different ($p \leq 0.05$).

The antioxidant ability of stirred yogurt samples colored with turmeric and curcumin extracts (Table 5) indicated to the potent antioxidant properties of the turmeric and its curcumin. Moreover, there is a significant difference between the turmeric extract and curcumin extract; which meaning that many other compounds besides curcumin contribute to antioxidant properties of the turmeric. These findings were in agreement with that of Dave et al. [38] who reported that fat-and water-soluble extracts of turmeric and its curcumin component had strong antioxidant activity, comparable to vitamins E and C.

The effect of coloring stirred yogurt with red cabbage and turmeric extracts on the total phenolic content (TPC) is shown in Table 5. By the same trend of RSA values; the addition of color extracts increased significantly ($p \leq 0.05$) the content of TPC in all treatments. However, the red cabbage juice was insignificant higher than anthocyanin extract.

It is obvious also that the treatment of turmeric extract was significantly ($p \leq 0.05$) the highest in its content of TPC than control and red cabbage extracts; and there is a significant different between the turmeric extract and curcumin extract. Finally, the obtained results

of TPC clarified a linear relationship between antioxidant capacity and phenolic contents in the all treatments.

Sensory properties of colored stirred yogurt

Table 6 illustrated the sensory attributes, intent to purchase and the acceptability index for all treatments. All the formulated colored stirred yogurts showed accepted scores higher than 7 in all attributes which indicated that the panelists liked all the yogurt products. The recorded scores for flavor were significantly lower in turmeric and curcumin extracts than red cabbage extracts and control; this can be due to the strong flavor of turmeric. While red cabbage extracts flavor is faint; so did not affect the flavor of stirred yogurt. The color scores were acceptable for all treatments than the control (plain without color) without significant differences between them. The color of stirred yogurt with anthocyanin extract was violet-red; which was the most acceptable color than all samples. Likewise, the color of stirred yogurt with red cabbage was slightly purple, and the colors of turmeric treatments were yellow. As for texture and appearance scores, the treatments of turmeric and curcumin were less acceptable than control and red cabbage samples; this may be related to incomplete the solubility of turmeric or curcumin in stirred yogurt and this affected the appearance of samples.

Parameter	Treatments				
	Yogurt Control (plain stirred)	Stirred yogurt colored with 10% red cabbage juice	Stirred yogurt colored with 10% aqueous extracts of anthocyanin	Stirred yogurt colored with 10% aqueous extracts of turmeric	Stirred yogurt colored with 10% aqueous extract of curcumin
Flavor (1-9)	8.9 ^a	8.4 ^a	8.6 ^a	7.1 ^b	7.5 ^b
Color (1-9)	8.0 ^a	8.6 ^a	8.9 ^a	8.1 ^a	8.3 ^a
Texture (1-9)	8.6 ^a	8.4 ^a	8.5 ^a	7.0 ^b	7.2 ^b
Appearance (1-9)	8.0 ^a	8.9 ^a	8.9 ^a	7.1 ^b	7.4 ^b
Overall impression (1-9)	8.0 ^a	8.5 ^a	8.8 ^a	7.6 ^a	7.9 ^a
purchase intent (1-5)	-	4.6 ^a	4.9 ^a	4.0 ^a	4.2 ^a
Acceptability index (%)	-	88 ^b	95 ^a	75 ^c	78 ^c

Table 6: Sensory properties, purchase intent and acceptability of colored stirred yogurt. a,b,c: Values in the same row with different superscript small letters are statistically different ($p \leq 0.05$).

The scores of overall impression for all treatments were acceptable more than plain yogurt (control), which indicated to no causes for rejecting any sample.

The consumer is now very keen to eat healthy foods which are made from natural materials with health effects; so when the consumer knows the importance of the added substance to his health and his life the intent to purchase is increasing. The obtained scores for purchase intent showed that the red cabbage and anthocyanin treatments were the highest accepted products.

Regarding to the acceptance index, a product is considered accepted when the acceptability index achieved more than 70% [20].

So the stirred yoghurts colored with red cabbage and turmeric extracts reached the required standard. Finally, the preparation of stirred yogurt by adding extracts of red cabbage or turmeric are successful and acceptable.

Conclusions

The obtained results in this study could be concluded as follow:

Anthocyanins from red cabbage are polar pigments and high soluble in water than in non-polar solvents, so it can be easily prepared by using 100% distilled water.

The inclusions of red cabbage juice or anthocyanin extract in the stirred yogurt resulted in products with appropriate and acceptable physicochemical, microbiological and sensory attributes.

Anthocyanin in red cabbage is the most important dye and responsible for the color and antioxidant properties of red cabbage. In the same time, anthocyanin has a stimulating effect on the growth of lactic acid bacteria. So the extract of red cabbage anthocyanin is more useful to use in the preparation of stirred yogurt than the juice of red cabbage.

Turmeric and Curcumin are virtually insoluble in water at natural or acidic pH, so the obtained extracts were not completely clear.

The curcumin extract introduced colored stirred yogurt with somewhat good properties than the turmeric extract.

Using curcumin extract in preparing stirred yogurt is better than turmeric extract because it's slightly affected the growth of lactic acid bacteria and the rate of acidity in resultant yogurt.

To reduce the flavor of curcumin, it is recommended to use less than 10% of turmeric or curcumin extracts in preparing of stirred yogurt.

Finally, aqueous extract of anthocyanin from red cabbage is the best extract, followed by the curcumin extract. These extracts were best known for their health benefits for humans and recommended to use in stirred yogurt and another dairy products.

References

1. Abdeldaiem MH (2014) Use of yellow pigment extracted from turmeric (*Curcuma Longa*) rhizomes powder as natural food preservative. *American Journal of Food Science and Technology* 2: 36-47.
2. Friedrich M, Kuchlewska K (2013) Assessing the effect, on animal model, of mixture of food additives, on the water balance. *Acta Sci Pol, Technol Aliment*. 12: 81-90.
3. Chen Y, Wang Z, Zhang H, Liu Y, Zhang S, et al. (2018) Isolation of High Purity Anthocyanin Monomers from Red Cabbage with Recycling Preparative Liquid Chromatography and Their Photostability. *Molecules* 23: 991-1002.
4. Shipp J, Abdel-Aal EM (2010) Food applications and physiological effects of anthocyanins as functional food ingredients. *The Open Food Science Journal* 4: 7-22.
5. Presilski S, Presilaska N, Tomovska D (2016) Effects of extraction, conventional processing and storage on natural anthocyanins. *J Food Process Technol* 7: 551-553.
6. Khoo HE, Azlan A, Tang S, Tand Lim SM (2017) Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research* 61: 1-21.
7. Mathew D, Hsu W (2018) Antiviral potential of curcumin. *J Functional Foods* 40: 692-699.
8. Hosny IM, El Kholy WI, Murad HA, El Dairouty RK (2011) Antimicrobial activity of Curcumin upon pathogenic microorganisms during manufacture and storage of a novel style cheese 'Karishcum'. *Journal of American Science* 7: 611-618.
9. Richart SM, Yi Lin L, Mizushina Y, Chang YY, Chung TY, et al. (2018) Synergic effect of curcumin and its structural analogue

- (Monoacetylcurcumin) on anti-influenza virus infection. *J Food and Drug Analysis* 26: 1015-1023.
10. Sahat NS, Abang Zaidel DN, Muhamad II, Alam H Z (2014) Stability study of water-in-Oil emulsion containing anthocyanin from red cabbage. *Journal Teknologi (Sciences & Engineering)* 69: 1-5.
 11. Ibrahim J, Abdel hakim EH (2015) Quality improvement of damietta cheese using some spices extract. *Assiut Vet Med J* 61: 24-32.
 12. Mosiyani ZG, Pourahmad R, Eshaghi MR (2017) Investigating the effect of aqueous extracts of basil and savory on antioxidant activity, microbial and sensory properties of probiotic yogurt. *Acta Sci Pol Technol Aliment* 16: 311-320.
 13. Rizk EM, Azouz A, Lobna AM Hareedy (2009) Evaluation of red cabbage anthocyanin pigments and its potential uses as antioxidant and natural food colorants. *Arab Univ J Agric Sci., Ain Shams Univ. Cairo* 17: 361-372.
 14. Geethanjali A, Lalitha P, Jannathul FM (2016) Analysis of curcumin content of turmeric samples from various states of india. *International Journal of Pharma and Chemical Research* 2: 55-62.
 15. Ahmadiani N, Robbins RJ, Collins TM, Giusti MM (2014) Anthocyanins contents, profiles, and color characteristics of red cabbage extracts from different cultivars and maturity stages. *J Agric Food Chem* 62: 7524-7531.
 16. AOAC (2012) Official methods of analysis. Association of Official Analytical chemists, 19th Ed.), Gaithersburg, Maryland, USA, 20877-2417, USA.
 17. Wehr HM, Frank JF (2004) Standard methods for the examination of dairy products. (17thEd.). American Public Health Association, Washington DC, USA.
 18. Gulluce M, Sokmen M, Sahin F, Sokmen A, Adiguzel A, et al. (2004) Biological activities of the essential oil and methanolic extract of *Micromeria fruticosa* (L) Druce ssp *Serpy llifolia* (Bieb) PH Davis plants from the eastern anatolia region of turkey. *Journal of the Science of Food and Agriculture* 84: 735-741.
 19. Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem* 49: 5165-5170.
 20. Januário JG, Da Silva IC, De Oliveira AS, De Oliveira JF, Dionísio JN, et al. (2017) Probiotic yoghurt flavored with organic beet with carrot, cassava, sweet potato or corn juice: Physicochemical and texture evaluation, probiotic viability and acceptance. *Int Food Res J* 24: 359-366.
 21. SPSS (2007) Statistical package for social science. SPSS Inc., 233 South Wacker Drive, Chicago, USA.
 22. Xavier MF, Lopes TJ, Quadri MG, Quadri MB (2008) Extraction of red cabbage anthocyanins: Optimization of the Operation Conditions of the Column Process. *Braz arch biol technol* 51: 143-152.
 23. Wu XL, Beecher GR, Holden JM, Haytowitz DB (2006) Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *J Agric Food Chem* 54: 4069-4075.
 24. Alghamdi E (2013) The intake of red cabbage anthocyanines in ice-cream. *Life Sci J* 9: 2654-2657.
 25. Surojanametakul V, Satmalee P, Saengprakai J, Siliwan D, Wattanasiritham L (2010) Preparation of curcuminoid powder from turmeric root (*Curcuma longa* Linn) for food ingredient use. *Kasetsart J (Nat Sci)* 44: 123-130.
 26. Asghari G, Mostajeran A, Shebli M (2009) Curcuminoid and essential oil components of turmeric at different stages of growth cultivated in Iran. *Research in Pharmaceutical Sciences* 4: 55-61.
 27. Dhakal S, Chao K, Schmidt W, Qin J, Kim M, et al. (2016) Evaluation of turmeric powder adulterated with metanil yellow using FT-Raman and FT-IR Spectroscopy. *Foods* 5: 36-50.
 28. Pawar HA, Gavasane AJ, Choudhary PD (2018) A Novel and simple approach for extraction and isolation of curcuminoids from turmeric rhizomes. *Nat Prod Chem Res* 6: 300-303.
 29. De Lima MS, Pereira MA, Plácido GR, Cagnin C, Vieira NF, et al. (2017) Physical and chemical characteristics and drying kinetics of turmeric (*Curcuma longa* L.). *African Journal of Agricultural Research* 12: 28-34.
 30. Damunupola DA, Weerathilake WA, Sumanasekara GS (2014) Evaluation of quality characteristics of goat milk yogurt incorporated with beetroot. *Juice International Journal of Scientific and Research Publications* 4: 1-5.
 31. Dimitrovski D, Stojanovska MC, Simonovska J, Doneva Sapceska D (2016) Lactic acid fermentation of cabbage juice by probiotic strain. III International Congress "Food Technology, Quality and Safety", pp: 67-73.
 32. Fu S, Augustin MA, Shen Z, Sanguansri L, Ajlouni S (2015) Bioaccessibility of curcuminoids in buttermilk in simulated gastrointestinal digestion models. *Food Chem* 15: 179: 52-59.
 33. Lucas A, Sodini I, Monnet C, Jolivet P, Corrieu G (2004) Probiotic cell counts and acidification in fermented milks supplemented with milk protein hydrolysates. *Int Dairy J* 14: 47-53.
 34. Seelee W, Tungjaroenchai W, Natvaratat M (2009) Development of low fat set-type probiotic yoghurt from goat milk. *Asian Journal of Food and Agro-Industry* 2: 771-779.
 35. Semjonovs P, Shakizova L, Denina I, Kozlinskis E, Unite D (2014) Development of a fructan-supplemented synbiotic cabbage juice beverage fermented by *Bifidobacterium lactis* Bb12. *Research Journal of Microbiology* 9: 129-141.
 36. Rahmatalla SA, Abd Alazeem L, Abdalla MO (2017) Microbiological quality of set yoghurt supplemented with turmeric powder (*Curcuma longa*) during storage. *Asian Journal of Agriculture and Food Sciences* 5: 1-6.
 37. Gjorgievski N, Tomovska J, Dimitrovska G (2014) Determination of the antioxidant activity in yogurt. *J. Hygienic Engineering and Design* 8: 88-92.
 38. Dave S, Vijay S, Keswani H, Sharma S (2017) Curcumin-a magical medicine: A comprehensive review. *International Ayurvedic Medical Journal* 5: 458-467.